# DESIGN OF A CHIMERIC ANTIFREEZE PROTEIN FOR BACTERIAL STOCKS PRESERVATION

Universitat Autònoma de Barcelona

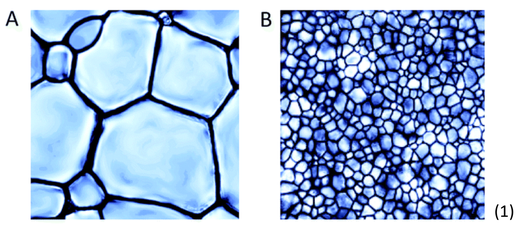
Jessica Angulo Capel

**This is a research project aimed at the design of a protein for its application as a cryoprotectant in bacterial stocks, as an alterative to glycerol, whose long term cytotoxicity has been demonstrated.**

## BACKGROUND AND SIGNIFICANCE

### ANTIFREEZE PROTEINS

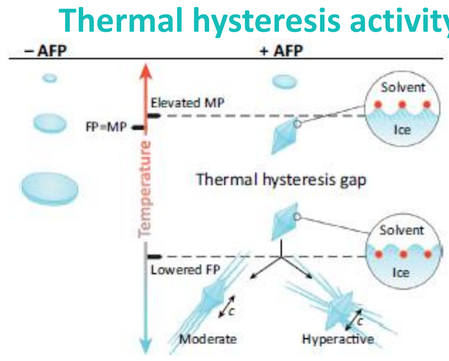
#### Ice recrystallization inhibition (IRI)




(1)

Ice recrystallization is the process by which large crystals grow at the expense of small ones.

#### Thermal hysteresis activity (TH)



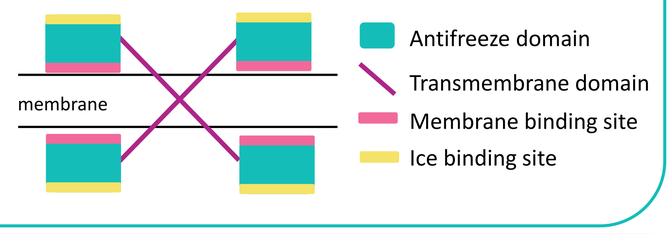

FP = freezing point
MP = melting point

(2)

### PROJECT PURPOSE

- Ice recrystallization is the main cause of cell damage during cryopreservation.
- Ice recrystallization inhibition activity prevents this process, while thermal hysteresis tends to generate an ice crystal with sharp ends.
- Cell preservation studies have been performed with soluble AFPs, which can't prevent intracellular ice formation.

**Aim of the project: Design of a new AFP with a high IRI and a low TH activity. It will also be able to protect cell membrane from intracellular and extracellular ice crystals.**




## METHODOLOGY

### PRIMARY SEQUENCE DESIGN

After analysing 11 AFP, the main candidates were:

wfAFP (Type I AFP)

- ✓ Soluble (phage display)
- ✓ Small size (phage display)
- ✗ Non-defined ice binding site

LpAFP

- ✓ Soluble (phage display)
- ✗ Big size (phage display)
- ✓ Defined ice binding site
- ✗ Low stability at 25 and 0ºC (measured with FoldX)

ΔG(25ºC) = 35.01kcal/mol
ΔG(0ºC) = 26.01kcal/mol

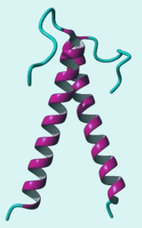
#### Chosen AFP

LeIBP

- ✓ Soluble (phage display)
- ✗ Big size (phage display)
- ✓ Defined ice binding site
- ✓ High stability at 25 and 0ºC (measured with FoldX)

ΔG(25ºC) = -12.61.01kcal/mol
ΔG(0ºC) = -33.76kcal/mol

#### Chosen transmembrane region



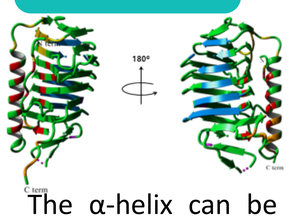
**Glycophorin A**

This protein has been used as a fusion with an antibiotic protein and the construct was correctly expressed in E. coli.

### MEMBRANE BINDING SITE DESIGN

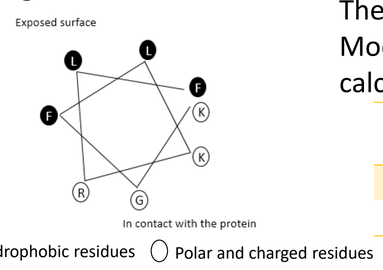
#### In silico approach

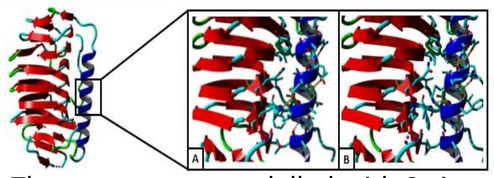
**Identification of important residues** → **Design of an amphipatic α-helix** → **Introduction of the MTS into LpIBP's α-helix** → **Model of the mutated protein and stability analysis**




The membrane targeting sequence, MTS (from the protein MinD) has shown to be a transplantable lipid-binding motif

The α-helix can be a good region to be mutated as we can create an amphipatic α-helix which can be located in membrane's interphase



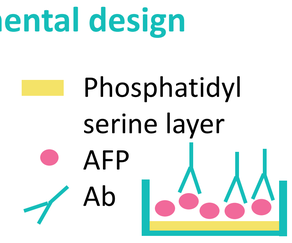

The mutant was modelled with Swiss-Model and the stability was calculated with FoldX

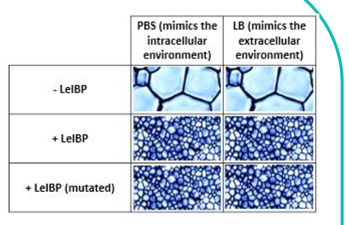
| | ΔG (kcal/mol) Before | ΔG (kcal/mol) After |
|---|---|---|
| 25ºC | -5.52 | -12.61 |
| 0ºC | -26.02 | -33.76 |

**The mutation is stabilizing!**

#### Experimental design

It will be used saturation mutagenesis, where a single codon for a particular amino acid is mutated by the other 19 amino acids.




**Semi-rational mutagensis on the α-helix** → **Phage display** → **PS-binding Enzyme-linked Immunosorbent Assay (ELISA)** → **IRI activity (Splat-cooling assay)**

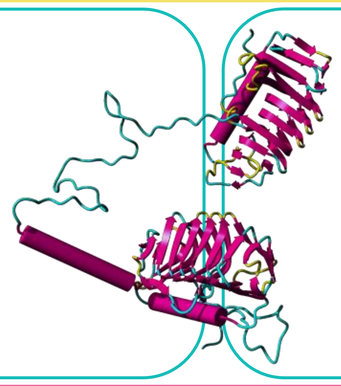
The protein will be displayed fused to the pIII coating protein of bacteriophage M13.
It will also be used the phagemid system.

Sample solution is frozen as a thin circular wafer
Within a cryostage held at -6ºC, it will be photographed using a digital camera inside a microscope

## FINAL DESIGN ANALYSIS

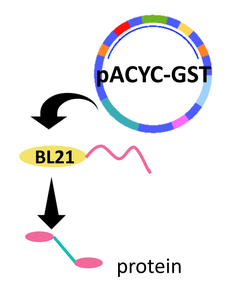
#### In silico testing

- **Secondary structure prediction (Quick2D)** → Conserved
- **Topology prediction (Membrane Protein Explorer)** → 3 TM regions (2 within the AFP)
- **Aggregation (Aggrescan)** → 23 small hot spots spread over the whole protein
- **Stability prediction (FoldX)** → ΔG (25ºC) = 766.82kcal/mol
  ΔG (0ºC) = 713.72kcal/mol



#### Experimental testing

- **Protein expression** → Western Blot
- **Topology analysis** → Liposome → degradation
- **IRI activity** → Splat-cooling assay




## WORKING PLAN

### CHRONOGRAM

| Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Membrane binding site design | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | | | | | | | | | | | | |
| Semi-rational mutagenesis | ■ | ■ | ■ | | | | | | | | | | | | | | | | | | | | | |
| Phage display | | | | ■ | ■ | ■ | ■ | ■ | | | | | | | | | | | | | | | | |
| PS-binding ELISA | | | | ■ | ■ | ■ | ■ | ■ | | | | | | | | | | | | | | | | |
| IRI activity assay | | | | | | | | | ■ | ■ | ■ | | | | | | | | | | | | | |
| 1rst phase results analysis | | | | | | | | | | | | ■ | | | | | | | | | | | | |
| Final design analysis | | | | | | | | | | | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Protein expression | | | | | | | | | | | | | ■ | ■ | ■ | | | | | | | | | |
| Topology analysis | | | | | | | | | | | | | | | | ■ | ■ | ■ | | | | | | |
| IRI activity assay | | | | | | | | | | | | | | | | | | | ■ | ■ | | | | |
| 2nd phase results analysis | | | | | | | | | | | | | | | | | | | | | ■ | ■ | | |

### BUDGET AND STAFF

| | Annual Cost (€) | Total Amount (€) |
|---|---|---|
| SALARIES AND WAGES | 54,000.00 | 108,000.00 |
| Research assistants | 36,000.00 | 72,000.00 |
| Technicians | 18,000.00 | 36,000.00 |
| EQUIPMENT | 8,200.00 | 16,400.00 |
| Office equipment | 200.00 | 400.00 |
| Equipment installation | 8,000.00 | 16,000.00 |
| ANALYTICAL/SCIENTIFICAL SERVICES | 50,000.00 | 100,000.00 |
| Publication costs | 1,000.00 | 2,000.00 |
| Material | 49,000.00 | 98,000.00 |
| TRAVEL | 14,000.00 | 28,000.00 |
| Professional meetings | 8,000.00 | 416,000.00 |
| Travel for consultation | 6,000.00 | 12,000.00 |
| INDIRECT COSTS | 45,000.00 | **90,000.00** |
| | | **342,400.00** |

The personal will consist in two research assistants and one technician.

## DISCUSSION

- What is new from other studies is that a new AFP has been designed and optimized for having the appropriate features which are needed to be a good cryoprotectant.
- The results will be very useful for future projects focused in the design of a new bacterial strand because they will provide with a lot of information regarding the protein expression, topology and stability, as well as in vivo capacity to prevent freeze damage.
- We will have to deal with the typical problems we find when working with new engineered proteins. F. ex. Stability, correct folding, topology…

## BIBLIOGRAPHY

(1) Davies, P. L. Ice-binding proteins: A remarkable diversity of structures for stopping and starting ice growth. *Trends Biochem. Sci.* **39,** 548–555 (2014).

(2) Balcerzak, A. K., Capicciotti, C. J., Briard, J. G. & Ben, R. N. Designing ice recrystallization inhibitors: from antifreeze (glyco)proteins to small molecules. *RSC Adv.* **4,** 42682–42696 (2014).